

PRELIMINARY AMENDMENT
New U.S. National Stage Entry of PCT/JP2004/012107

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions and listings of claims in the application:

LISTING OF CLAIMS:

1. (Original) A method of analyzing the C-terminal amino acid sequence of the peptide, comprising:
obtaining C-terminal-deleted peptides lacking amino acid residues from said C-terminal by degrading the amino acids from said peptide C-terminal sequentially;
measuring the molecular weight of said C-terminal-deleted peptides; and
determining the decrease in molecular weight associated with said sequential degradation from the difference between the molecular weight obtained in said measuring the molecular weight of the C-terminal-deleted peptides and the molecular weight of said peptide, and
analyzing said C-terminal amino acid sequence based on the decrease in said molecular weight, wherein said C-terminal amino acids are degraded by making said peptide substantially bring into contact with an alcanoic anhydride in said obtaining C-terminal-deleted peptides.

2. (Original) The method of analyzing the C-terminal amino acid sequence of the peptide according to Claim 1, further comprising measuring the molecular weight of said peptide, wherein said analyzing the amino acid sequence enables the decrease in molecular weight associated with the sequential degradation to be determined from the difference between the molecular weight obtained in said measuring the molecular weight of the peptide and the

PRELIMINARY AMENDMENT

New U.S. National Stage Entry of PCT/JP2004/012107

molecular weight obtained in said measuring the molecular weight of C-terminal-deleted peptides.

3. (Currently Amended) The method of analyzing the C-terminal amino acid sequence of the peptide according to claim 1 ~~Claim 1 or 2~~, further comprising allowing water molecules to act on said C-terminal-deleted peptides after said obtaining the C-terminal-deleted peptides and before said measuring the molecular weight of the C-terminal-deleted peptides.

4. (Original) The method of analyzing the C-terminal amino acid sequence of the peptide according to Claim 3,

wherein said allowing water molecules to act includes bringing said C-terminal-deleted peptides into contact with an aqueous solution containing a basic nitrogen-containing compound or a tertiary amine.

5. (Original) A method of analyzing the C-terminal amino acid sequence of the peptide, comprising:

obtaining C-terminal-deleted peptides lacking amino acid residues from said C-terminal by degrading the amino acids from said C-terminal of said peptide sequentially;

obtaining C-terminal-deleted peptide-derived peptide fragments by cleaving said C-terminal-deleted peptides at predetermined positions;

measuring the molecular weight of said C-terminal-deleted peptide-derived peptide fragments;

PRELIMINARY AMENDMENT

New U.S. National Stage Entry of PCT/JP2004/012107

determining the decrease in molecular weight associated with said sequential degradation from the difference between the molecular weight obtained in said measuring the molecular weight of C-terminal-deleted peptide-derived peptide fragments and the molecular weight of the peptide fragments obtainable from said peptide and analyzing said C-terminal amino acid sequence based on said decrease in molecular weight,

wherein said C-terminal amino acids are degraded by making said peptide substantially bring into contact with an alcanoic anhydride in said obtaining C-terminal-deleted peptides.

6. (Original) The method of analyzing the C-terminal amino acid sequence of the peptide according to Claim 5, further comprising:

obtaining peptide-derived peptide fragments by cleaving said peptide at said predetermined positions; and

measuring the molecular weight of said peptide-derived peptide fragments,

wherein the decrease in molecular weight associated with said sequential degradation is determined from the difference between the molecular weight obtained in said measuring the molecular weight of the peptide-derived peptide fragments and the molecular weight obtained in said measuring the molecular weight of C-terminal-deleted peptide-derived peptide fragments.

7. (Currently Amended) The method of analyzing the C-terminal amino acid sequence of the peptide according to claim 5 ~~Claim 5 or 6~~,

wherein said obtaining the C-terminal-deleted peptides includes protecting particular amino acid residues in said peptide and thus eliminating the susceptibility of said particular

PRELIMINARY AMENDMENT

New U.S. National Stage Entry of PCT/JP2004/012107

amino acid residues to said cleavage in said obtaining the C-terminal-deleted peptide-derived peptide fragments.

8. (Original) The method of analyzing the C-terminal amino acid sequence of the peptide according to Claim 7,

wherein said obtaining the C-terminal-deleted peptide-derived peptide fragments includes treating said C-terminal-deleted peptides with a protease.

9. (Original) The method of analyzing the C-terminal amino acid sequence of the peptide according to Claim 8,

wherein said protease is trypsin, and said eliminating the susceptibility of the particular amino acid residues includes N-acylating said peptide.

10. (Original) The method of analyzing the C-terminal amino acid sequence of the peptide according to any one of Claims 7 to 9,

wherein said protection is O- and N-acylation of said peptide and the protecting groups by O-acylation are deprotected after said obtaining the C-terminal-deleted peptide and before said obtaining the C-terminal-deleted peptide-derived peptide fragments.

11. (Currently Amended) The method of analyzing the C-terminal amino acid sequence of the peptide according to claim 5 any of Claims 5 to 10,

PRELIMINARY AMENDMENT

New U.S. National Stage Entry of PCT/JP2004/012107

wherein said measuring the molecular weight of C-terminal-deleted peptide-derived peptide fragments includes performing mass spectrometric measurement based on cationic and anionic species; and

said analyzing the amino acid sequence from the C-terminal includes identifying said C-terminal-deleted peptide-derived peptide fragments associated with said C-terminal of said peptide by comparing the mass spectrometric results based on cationic species and the mass spectrometric results based on anionic species.

12. (Currently Amended) The method of analyzing the C-terminal amino acid sequence of the peptide according to claim 5 any of Claims 5 to 11, further comprising allowing water molecules to act on said C-terminal-deleted peptides after said obtaining the C-terminal-deleted peptides and before said obtaining the C-terminal-deleted peptide-derived peptide fragments.

13. (Original) The method of analyzing the C-terminal amino acid sequence of the peptide according to Claim 12,

wherein said allowing water molecules to act includes bringing said C-terminal-deleted peptides into contact with an aqueous solution containing a basic nitrogen-containing aromatic ring compound or a tertiary amine.

14. (Currently Amended) The method of analyzing the C-terminal amino acid sequence of the peptide according to claim 5 any one of Claims 5 to 13,

PRELIMINARY AMENDMENT

New U.S. National Stage Entry of PCT/JP2004/012107

wherein said obtaining the C-terminal-deleted peptides is carried out while said peptide is retained in the gel.

15. (Currently Amended) The method of analyzing the C-terminal amino acid sequence of the peptide according to claim 1 any of Claims 1 to 4,

wherein the processings prior to said measuring the molecular weight of the C-terminal-deleted peptides are performed in the gel.

16. (Currently Amended) The method of analyzing the C-terminal amino acid sequence of the peptide according to claim 5 any of Claims 5 to 13,

wherein the processings prior to said measuring the molecular weight of C-terminal-deleted peptide-derived peptide fragments are performed in the gel.

17. (Currently Amended) The method of analyzing the C-terminal amino acid sequence of the peptide according to claim 14 any of Claims 14 to 16, further comprising crosslinking said peptide before said obtaining the C-terminal-deleted peptides.

18. (Currently Amended) The method of analyzing the C-terminal amino acid sequence of the peptide according to claim 14 any of Claims 14 to 17, further comprising isolating said peptide from the peptide-containing mixture by polyacrylamide gel electrophoresis before said obtaining the C-terminal-deleted peptides,

PRELIMINARY AMENDMENT

New U.S. National Stage Entry of PCT/JP2004/012107

wherein said obtaining the C-terminal-deleted peptides is performed while said isolated peptide is retained in said gel used in said polyacrylamide gel electrophoresis.

19. (Currently Amended) The method of analyzing the C-terminal amino acid sequence of the peptide according to claim 14 any of Claims 14 to 18,

wherein said obtaining the C-terminal-deleted peptides includes immersing said gel in a solution of an alkanoic anhydride in a dipolar aprotic solvent.

20. The method of analyzing the C-terminal amino acid sequence of the peptide according to claim 1 any of Claims 1 to 19,

wherein said obtaining the C-terminal-deleted peptides is carried out in a system containing a basic nitrogen-containing aromatic ring compound.

21. (Original) The method of analyzing the C-terminal amino acid sequence of the peptide according to Claim 20,

wherein said basic nitrogen-containing aromatic ring compound is a pyridine base or the derivative of the pyridine base.

22. (Currently Amended) The method of analyzing the C-terminal amino acid sequence of the peptide according to claim 1 any of Claims 1 to 21,

wherein said alkanoic anhydride is the symmetric anhydride of an alkanoic acid of 2 or more and 6 or less carbon atoms.

PRELIMINARY AMENDMENT
New U.S. National Stage Entry of PCT/JP2004/012107

23. (Currently Amended) The method of analyzing the C-terminal amino acid sequence of the peptide according to claim 1 any of Claims 1 to 22,

wherein said alkanoic anhydride is the symmetric anhydride of a straight-chain alkanoic acid of 2 or more and 6 or less carbon atoms.